IN THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (previously presented) A method for determining a characteristic kinetic quantity of a chemical reaction in a sample

wherein said chemical reaction involves a plurality of chemical species, at least a first one of said species including a fluorophore being a FRET acceptor of a FRET pair consisting of a FRET donor and a FRET acceptor and a at least a second one of said species including a fluorophore being a FRET donor of said FRET pair,

said acceptor being a photochrome, the absorption spectrum of which being changeable by irradiation with light of a suitable wavelength, and

said donor being a fluorophore, the emission spectrum of which having an overlap region with said FRET acceptor's absorption spectrum, the size of said overlap region being dependent on the photochromic state of said FRET acceptor

wherein said chemical reaction reversibly converts said first and second species as free ligands into at least one product comprising a combination of said first and second species, the method comprising the steps of:

having light of a wavelength capable of switching said photochromic state of said FRET acceptor impinge on said sample with the chemical reaction being in its equilibrium state, thereby switching said photochromic state of said acceptor in said product of said chemical reaction less efficiently than in said free ligand, thus generating a non-equilibrium state of said chemical reaction, and

observing, by means of a FRET dependent fluorescence signal of at least one of said fluorophore and said acceptor, at least one temporal portion of a relaxation of concentrations of said species involved.

2. (previously presented) A method according to claim 1, wherein the fluorescence of said FRET donor is measured in order to observe said relaxation.

3. (previously presented) A method according to claim 1, wherein said photochromic FRET acceptor is a fluorophore and wherein the fluorescence of said photochromic FRET acceptor is measured in order to observe said relaxation.

4. (previously presented) A method according to claim 1, wherein the product under test comprises an additional fluorophore which represents an additional FRET acceptor to said FRET donor.

5. (previously presented) A method according to claim 4, wherein said additional FRET acceptor is no photochrome.

6. (previously presented) A method according to claim 4, wherein the fluorescence of said additional FRET acceptor is measured in order to observe said relaxation.

7. (previously presented) A method according to claim 1, wherein a change in the photochromic state of said FRET acceptor in a first direction is caused by irradiation of said sample with light of a first wavelength and wherein a change in the photochromic state of said FRET acceptor in a second direction is caused by irradiation of said sample with light of a second wavelength.

8. (previously presented) A method according to claim 1, wherein said change in said

photochromic state of said FRET acceptor in at least one direction is caused by irradiation with ultraviolet light.

9. (previously presented) A method according to claim 1, wherein said change in said photochromic state of said FRET acceptor in at least one direction is caused by irradiation with visible light.

10. (previously presented) A method according to claim 1, wherein said excitation of said FRET acceptor is caused by irradiation with visible light.

11. (previously presented) A method according to claim 1, wherein the intensity of irradiation used to change said photochromic state of said FRET acceptor is substantially stronger than the intensity of irradiation used to generate the observed fluorescence.

12. (previously presented) A method according to claim 1, wherein said sample is irradiated in a temporally modulated fashion in order to change said photochromic state of said FRET acceptor.

13. (previously presented) A method according claim 7, wherein said sample is irradiated with light of said first wavelength and said second wavelength in an alternating fashion in order to change said photochromic state of said FRET acceptor.

14-16. (canceled)

17. (new) A method for determining a reaction rate of a reversible chemical binding reaction between a first chemical species and a second chemical species in a sample, the method comprising:

providing said first chemical species including a first fluorophore being a FRET acceptor of a FRET pair consisting of a FRET donor and a FRET acceptor,

proving said second chemical species including a second fluorophore being a FRET donor of said FRET pair, said FRET donor being excitable by means of irradiation with light of a suitable excitation wavelength,

wherein said FRET acceptor is a photochromic FRET acceptor, which is switchable - by mean of irradiation with light of a suitable switching wavelength - from an OFF-state, where its absorption spectrum overlaps the emission spectrum of said FRET donor to a minor extent, to an ON-state, where its absorption spectrum overlaps said emission spectrum of said FRET donor to a major extent,

mixing said first and said second chemical species in said sample under thermodynamical conditions that allow the establishment of a dynamic equilibrium between a free state, where said first and said second chemical species are free ligands, and a bound state, where said first and said second chemical species are bound to each other in a complex, such that in said complex said FRET donor and said FRET acceptor are positioned closely enough to allow energy transfer,

irradiating said sample by light of said excitation wavelength, thereby exciting said FRET donor, and observing - in a time- resolved manner - the fluorescence signal of said FRET donor an/or said FRET acceptor,

irradiating said sample by a flash of light of said switching wavelength, thereby switching said acceptor from said OFF-state to said ON-state, wherein the process of switching is less efficient in said bound state compared to said free state due to an additional FRET channel of de-excitation which is available in said bound state and is not available in said free state,

calculating said reaction rate from the exponential development of said fluorescence signal.

18. (new) A method for determining a reaction rate of a dynamic reversible chemical binding reaction between a first molecule species (R1) and at least a second molecule species (R2) in a sample, the method comprising:

(a) labelling said first molecule (R1) with a fluorophore;

(b) labelling said second molecule (R2) with a fluorophore (F2) or chromophore (C2),

wherein

- the fluorophore (F1) labelling said first molecule (R1) is a FRET donor of a FRET pair consisting of a FRET donor and a FRET acceptor,
- the FRET donor is excitable to fluoresce at an emission wavelength upon irradiation with light of an excitation wavelength,
- the second fluorophore (F2) or chromophore (C2) is a FRET acceptor of the FRET pair,
- the FRET acceptor is a photochromic FRET acceptor, which is switchable from an OFF-state to an ON-state by mean of irradiation with light of an ON switching wavelength, wherein in the OFF-state its absorption spectrum overlaps the emission spectrum of said FRET donor to a minor extent, and in the ON-state its absorption spectrum overlaps said emission spectrum of said FRET donor to a major extent;

(c) mixing said labelled first molecule species and said labelled second molecule species in said sample under thermodynamic conditions that allow the establishment of a pre-switching dynamic (forward and reverse reaction of complex formation) equilibrium between

- an unbound state, where said first and said second molecular species are free ligands, and
- a bound state, where said first and said second molecular species are bound to each other in a complex, such that in said complex said FRET donor and said FRET acceptor are positioned closely enough to allow energy transfer;

(d1) irradiating said sample by light of said excitation wavelength, thereby exciting said FRET donor;

(d2) recording time vs. intensity of the fluorescence signal of said FRET donor an/or said FRET acceptor, wherein donor flouresence indicates free ligands and acceptor fluorescence indicates bonding;

(e) irradiating said sample by a flash of light of said ON switching wavelength, thereby switching said acceptor in at least some of said second molecule species from said OFF-state to said ON-state, producing a mixture comprising: (1)free molecule with acceptor in the ON-state, (2) free molecule with acceptor in the OFF-state, (3)bound molecule with acceptor in the ON-state, (4) bound molecule with acceptor in the OFF-state,

wherein said acceptor in the ON state relaxes to OFF state, and wherein said relaxation is facilitated to a greater extent in said bound molecules, as compared to free acceptor in the ON-state, due to the presence of a FRET channel of excitation in the bound molecules, thereby creating a non-equilibrium state by an underpopulation of bound acceptor in the ON-state as compared to free acceptor in the ON-state;

(g1) irradiating said sample by light of said excitation wavelength, thereby exciting said FRET donor;

(g2) recording time vs. intensity of the fluorescence signal of said FRET donor and/or said FRET acceptor during relaxation from non-equilibrium to equilibrium; and

(h) calculating said reaction rate from the exponential development of said fluorescence signal.